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## The uptake of cations by vallisneria leaves

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rest of the protoplasm. It gives the picture of a simple unperforated membrane of 50–100 Å thick, which is not of the same type as the double membranes of plastids, protoplastids or chondriosomes. This worker considers the plasmalemma as an artefact. One should keep in mind, however, that a difference in structure does not implicate a difference in physiological behaviour, so that it is not allowed to conclude that such an outer boundary is also a barrier to free diffusion of ions. BUVAT and LANCE (1957) showed electron-microscopically that the cytoplasm of leaves of various plants is bordered by a double membrane. It is known that this kind of membranes generally forms barriers to the free diffusion of ions. In the previous section on the location of the fraction B in the plant cell, much evidence has been obtained in favour of a location of the total fraction B in the cell-wall. This implicates that the fraction A must also be restricted to the cell-walls, for otherwise a part of the fraction B would be located in the cytoplasm too, owing to the presence of immobile anions.

## CHAPTER IX

### GENERAL DISCUSSION

#### THE PRESENCE OF AN EXCHANGEABLE ANION FRACTION

In chapter II it has been shown that an anion fraction B, if present, must be very small. According to HOPE (1953) this is intelligible because at normal pH's the acid dissociation of amphoteric groups strongly predominates in plant cells. Also in literature there is much evidence for the existence of an exchangeable cation fraction in plant tissues but little for an exchangeable anion fraction (KYLIN and HYLMO, 1957; LEGGETT and EPSTEIN, 1956). This is in accordance with the present experiments. Yet there are some reports of an exchangeable anion fraction.

EPSTEIN (1955) found in barley roots a small labile bound  $\text{SO}_4$  fraction. Selenate ions proved to compete with the  $\text{SO}_4$  ions for the binding-sites and for this reason, this  $\text{SO}_4$  fraction has been considered to be due to adsorption-exchange (LATIES, 1959). Because it could be washed out in deionised water, we must accept that it is exchanged either for the hydroxyl ions, which are present in a very small concentration or for other anions in the medium. These anions may have diffused out of the plant tissue.

OVERSTREET and JACOBSON (1946) found in barley roots an exchangeable phosphate fraction but there was a marked difference in behaviour between the exchangeable Rb fraction and the exchangeable phosphate fraction in regard to their exchange for the unlabelled isotope. Whereas the Rb ions were efficiently removed, the phosphate ions were only slowly removed at a constant rate.

They placed the roots at a temperature of zero degree centigrade in a salt solution of the carrier-free iodine. By this the specific activity was very high and uptake and exchange of very small fractions

could be measured. The concentration of the iodide ions was very low, about  $10^{-9}$  M. Also here a marked difference in the behaviour of the anion and the cation was found. Whereas the uptake of Sr was very little affected by temperature and by killing the roots by ether, the uptake of the  $I^{-}$  was markedly reduced. Moreover the exchange of the absorbed iodide for the unlabelled isotope was very slow and almost linear to time in contrary to the normal exchange curve of an exchangeable cation fraction. Their conclusion was that the exchangeable iodide fraction is due to a metabolic uptake in contrast to the exchangeable Sr fraction, which is non metabolic. Anyhow, the nature of this exchangeable iodide fraction is quite different from the nature of the cation adsorption-exchange fraction and, therefore, cannot be compared with the latter.

LUNDEGÅRDH (1958) assumes that the "Initial Chloride Uptake" found in excised roots is based on adsorption and not on diffusion. This adsorption may be compared with the adsorption-exchange of cations. He claims that the fact that the initial absorption of chloride could be inhibited by KCN points to a subordinate role of pure diffusion in the initial uptake of salts. Lundegårdh, however, only determines differences in the concentration of the external solution before and after the uptake period. In this way adsorption-exchange cannot be demonstrated and experimental evidence for his conception was not obtained.

#### CARRIERS

As has already been mentioned in chapter I one of the theories about the mechanism of ion absorption by living cells is the carrier theory. This theory starts from the hypothesis that the ions are bound to some movable compound of the cytoplasm, which can pass a membrane impermeable to free ions. It may be asked whether or not the exchange sites to which the cations of fraction B are attached are identical with these carriers. By the experiments of several investigators EPSTEIN and HAGEN (1952), SCOTT and HAYWARD (1954), EPSTEIN and LEGGETT (1954), it has been shown that a very specific competition exists between ions of the same charge and between different groups of ions. Therefore, it is believed that special carriers react with particular ions. However, in *Vallisneria* there proved to be practically no specificity of the exchange sites for monovalent cations (chapter IV). This result is not in favour of the opinion that the exchange sites of fraction B are carriers in the above mentioned sense (LUNDEGÅRDH 1958).

A second objection is that the number of exchange sites is very large. This is in contrast with the generally accepted idea that carriers are present in very small amounts (HAGEN and HOPKINS, 1955; HAGEN, LEGGETT and JACKSON, 1957). The third objection is that the amount of anion carrier would be very small as opposed to the amount of the cation carriers. The most convincing proof against the supposition was obtained in the present experiments in which it

was demonstrated that the adsorption exchange (Fraction B) is not a necessary step in the metabolic uptake of the cations.

We may, therefore, safely assume that the adsorption-exchange in *Vallisneria* has nothing to do with reversible binding to carriers as described by the supporters of the carrier theory.

#### THE LOCATION OF THE EXCHANGEABLE FRACTION

It has been concluded in chapter viii that 96 percent of the La fraction B is located in the cell-wall. However, in calculating the percentage of the La fraction B in the cell-wall, the starting point was that the whole uptake of La-ions in *Vallisneria* leaves is non-metabolic, viz. fraction B. Though it has been discussed in chapter viii that a metabolic dependent La uptake, if present, must be very small, the possibility that a small metabolic dependent La uptake is present, can not be fully excluded. This possible error would decrease the calculated percentage of the La fraction B in the cell-wall. On the other hand we have seen that the La uptake in the living cell has not completely stopped after 24 hours. This may affect the result in the opposite direction. Both errors are small and they will not influence the result very much. Another objection against the conclusion that the La fraction B is in the cell-wall, may be based on the possibility that by homogenizing the tissue, the cell-wall is not completely separated from the cytoplasm. A measure for cytoplasm in the homogenate is its nitrogen content. The cytoplasm in the cell-wall after preparation is, at least partly, present in intact cells. These cells constitute a certain percentage of the total number originally present in the leaves. This percentage was determined by counting. It was approximately equal to the percentage of the nitrogen of the intact leaves that had remained in the cell-wall. This indicates that apart from the intact cells, little cytoplasm was present.

A second indication that the La fraction B is really located in the cell-wall, follows from the result, that the La fraction B in isolated cell-wall from plasmolysed cells has the same size as the La fraction B in isolated cell-wall from unplasmolysed cells. It is very unlikely that in plasmolysed cells the whole surface layer of the cytoplasm adheres to the cell-wall. Therefore, it is concluded that the exchange sites for the La ions are located in the cell-wall.

#### THE TRANSFER OF Ca FROM FRACTION B TO FRACTION C

In chapter vii it has been shown that the Ca-ions of the fraction B are hardly transferred to fraction C, whereas a metabolic Ca absorption occurred if the leaves were bathed in a salt solution of  $\text{CaCl}_2$ . This metabolic uptake of Ca into fraction C could be maintained for at least 24 hours. It may be asked why transfer of Ca from B to C does not take place, whereas Rb is readily transferred. As ions can be obtained from fraction B by exchange for other ions only, the transfer from B to C must depend upon exchange. It has been discussed in chapter iv that exchange of divalent cations of the D.F.S. for

monovalent cations is difficult, whereas the monovalent rubidium ions are readily exchanged for other monovalent ions. If this is true we may conclude that the transfer of ions from fraction B to fraction C depends upon exchange of these ions for monovalent cations (probably hydrogen ions) from the cell.

#### THE TRANSFER OF CA FROM FRACTION A TO FRACTION C

It was concluded from the experiments in chapter VII on the Ca uptake that the metabolic Ca uptake into the fraction C occurs directly from the external solution (fraction A) and not via the Ca fraction B. This has been considered to be a strong indication that the same holds for the Rb uptake into the fraction C.

One may wonder whether this is justified. In the following respect uptake of rubidium is similar to the uptake of calcium. The uptake into C of both ions is an active process, which may be inhibited by the same metabolic poisons. The fraction B of both cations is present in the cell-walls, since both may be exchanged for lanthanum and since 96 % of the lanthanum fraction B is located in the cell-walls. Neither a calcium fraction B nor a rubidium fraction B has been demonstrated to be present in the cytoplasm. This means that the active uptake into C takes place at the outer boundary of the cytoplasm. The only difference is that rubidium may be transferred from A to C and from B to C, whereas calcium is only transferred from A to C. There is no reason whatsoever to assume that a calcium fraction A may penetrate beyond the outer layer into the cytoplasm whereas a rubidium fraction A would stop at that layer. The fate of the two cations in the cell may be different, but it is not relevant to the question considered here. Whereas the Rb-ions are probably mainly accumulated into the vacuole (A. VAN SCHREVEN and A. VAN DER MOLEN, ARISZ in 1943, 1956) the Ca presumably remains in the cytoplasm (MAZIA, 1938), though it is also known of Ca ions that they may accumulated in the vacuole (CHASSON and LEVITT, 1957). We can therefore safely assume that the conclusion drawn from the experiments with  $\text{CaCl}_2$ , that the exchangeable fraction is not necessary for irreversible uptake, also holds for the Rb uptake into the fraction C.

#### THE LOCATION OF THE METABOLIC UPTAKE PROCESS IN THE CELL

From previous researches it has appeared that the uptake of chloride into the cytoplasm of *Vallisneria* leaves is an active process, using energy available in the cell or supplied when exposed to light (ARISZ, 1947, 1952, 1956). In chapter III it has been shown that the rate of the Rb uptake into the fraction C can strongly be inhibited by inhibitors of metabolic processes.

Moreover it was shown that the rate of uptake was dependent on temperature. From these results it may be concluded that the cation uptake into the fraction C is also only possible by an "active" accumulation mechanism. However, according to LUNDEGÅRDH (1945) the possibility remains that the rate of the cation accumulation in

cells is only inhibited by such factors as poisons, temperature etc., because the uptake of the accompanying anion is inhibited by these factors. The cation accumulation in it self may be a non-metabolic process, so that the cations are dragged passively with the anions, in order to maintain electrical neutrality within the cell. Whether the Rb-ions are passively dragged into the cells by anions which are taken up actively, or taken up actively is irrelevant to the problem discussed here.

In chapter v it was shown that not only the uptake of Rb into C is a metabolic process but also the transfer from B to C. It could be inhibited by monoiodoacetamide and it had a high temperature quotient. During the transfer of the Rb from fraction B to fraction C no added anions are available to join the Rb-ions. A fraction A has been washed out in the deionised water (chapter v) and for the anion a fraction B does not exist in measurable amounts (chapter II). Because in several experiments the pH of the deionised water was held at a pH 5, it is unlikely that measurable amounts of bicarbonate ions, that might join the Rb ions during the transfer to the fraction C, were present in the deionised water. These findings are strong indications that an uptake of cations exists which is independent of the uptake of anions. It follows that the cation uptake can directly be dependent on metabolism and that the outer boundary of the cytoplasm forms the barrier for a free diffusion of ions, which can only be passed at the expense of energy.

ARISZ demonstrated (1956) that cyanide has not a direct inhibiting influence on the secretion into the vacuole, but that it inhibits a process by which ions are actively absorbed into the cytoplasm. This shows that two different mechanism for the metabolic uptake of chloride are present in *Vallisneria* leaves. Arisz located one mechanism in the tonoplast and the other one somewhere in the cytoplasm. The present experiments do not permit any conclusion with regard to the accumulation mechanisms located at the tonoplast, but they furnish data on the existence of a mechanism regulating the absorption into the cytoplasm and moreover they allow conclusion about the place where this mechanism is located.

ROBERTSON *c.s.* (1955) assume that the accumulation mechanism is not located in the plasmalemma but in the outer boundary of mitochondria. According to ROBERTSON (1957) it is possible that these mitochondria move around by the protoplasmic streaming in the cell and that many of them frequently come into contact with the surface of the vacuole. It would be equally possible that they come into contact with the cell-wall, pick up ions here and lose them to the vacuole. However, it is known that in *Vallisneria* leaves the protoplasmic streaming comes to a standstill (JAGER, 1958) under conditions of high metabolic uptake and is not restored for the first few hours after bringing the leaves to deionised water. Thus a transfer of ions from wall to cytoplasm by means of mitochondria is not very likely. But even when it should take place, the first accumulation mechanism must be located at the boundary of cytoplasm and cell-wall.

## SUMMARY

1. Cations absorbed by *Vallisneria* leaves could be separated into three fractions, viz:

1. a fraction which was washed out in deionised water (fraction A)
2. a fraction which can be removed by exchange (fraction B)
3. a fraction which can be neither washed out nor exchanged.

2. Absorbed anions could be separated into two fractions only, viz: a fraction A and a fraction C; an exchangeable fraction being absent (chapter II).

3. The formation of the exchangeable fraction B depends on the physico-chemical and that of the fraction C on the biochemical properties of the plant (chapter III).

4. It is concluded that a Donnan equilibrium determines the exchangeable fraction B (chapter IV).

5. The transfer of rubidium ions from the exchangeable fraction B to fraction C is strongly affected by temperature and is inhibited by monoiodoacetamide (chapter V).

6. Of each number of rubidium ions accumulated as fraction B (exchangeable fraction) one part is more readily transferred to C than the rest. The rate of transfer of the first part is approximately linear to the number of ions present in B. The second portion is transferred at a much lower rate (chapter VI).

7. In contrast to exchangeable rubidium-ions, exchangeable calcium-ions of the fraction B are practically not transferred to fraction C, though Ca-ions are taken up in C from the external solution. This uptake depends on metabolism. It is concluded that adsorption-exchange in fraction B does not constitute a necessary link in the uptake of cations from the external solution into fraction C (chapter VII).

8. It is made probable that the exchangeable fraction (fraction B) is located in the cell-wall, and that the metabolic process which accumulates cations into the fraction C is located at the outer boundary of the cytoplasm. (Chapter VIII and General Discussion).

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